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בקשה לפטנט
Application For Patent

לשימוש הלשכה
For Office Use

מספר: Number	127346
תאריך: Date	01-12-1998
הוקדם/נדחה: Ante/Post-dated	

אני, (שם המבקש, מענו ולגבי גוף מאוגדת מקום התאגדותו)
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Method and system for detecting oligonucleotides in a sample

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דרישת דין קדימה*
Priority Claim

מדינת האיגוד Convention Country	תאריך Date	מספר/סימן Number/Mark

P.O.A.: General * יפוי כח: כללי
Filed in case P114692 הוגש בענין

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For the Applicants.
REINHOLD COHN AND PARTNERS
By: —

This 30 היום בחודש November שנת 1998
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שיטה ומערכת לזיהוי אוליגונוקליאודיטים בדגימה

Method and system for detecting oligonucleotides in a sample

Yissum Research Development
Company of the Hebrew
University of Jerusalem

C.113496.4

METHOD AND SYSTEM FOR DETECTING OLIGONUCLEOTIDES IN A SAMPLE

FIELD OF THE INVENTION

The present invention relates to a method and system for the detection of oligonucleotides in a sample.

PRIOR ART

The following is a list of prior art references which are relevant for a better understanding of the background of the invention:

1. Piunno, P.A.E., Krull, V.J., Hudson, R.H.E., Damha, M.J., Cohen, H., *Anal. Chim. Acta*, 288:205-209, (1994).

2. Mandenius, C.F., Chollet, A., Lenburg, M.M., Lundström, I., *Anal. Lett.*, 22:2961-2964, (1989).

3. Lidberg, B., Nylander, C., Lundström, I., *Sensors and Actuators*, 4:299-302, (1993).

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5. Mikkelsen, S.R., *Electroanalysis*, 8:15-23, (1996).

6. Millan, K.M., Sanauloo, A., Mikkelsen, S.R., *Anal. Chem.*, 66:3830-3833, (1994).

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8. Hashimoto, K., Ito, K., Ishimori, Y., *Anal. Chim. Acta*, **286**:219-224, (1994).

9. Wang, J., Palecek, E., Nielson, P.E., *J. Am. Chem. Soc.*, **118**:7667-7670, (1996).

10. Ihara, T., Nakayama, M., Murata, K., Maeda, M., *Chem. Commun.*, 1069-1070, (1997).

11. Bardea, A., Dagan, A., Ben-Dov, I., Amit, B., Willner, I., *Chem. Commun.*, 839-840, (1998).

12. PCT Application No. WO 97/04313.

Acknowledgement of these references in the description below will be made by indicating the number from the above list.

BACKGROUND OF THE INVENTION

The development of DNA-sensor devices attracts substantial recent research efforts directed to gene analysis, detection of genetic disorders, tissue matching and forensic applications. Optical detection of DNA was accomplished by the application of fluorescence labeled oligonucleotides[1,2] or by the use of surface plasmon resonance[3,4]. Electronic transduction of the formation of oligonucleotide complexes with a target DNA, and, particularly, in the quantitative assay of DNA is a major challenge of bioelectronics[5]. The organization of DNA-sensors requires the assembly of the sensing interface on a transducer, and the design of the appropriate electronic output that signals the formation of the recognition complex with the target DNA-analyte on the transducer element. Electrochemical DNA sensors based on the electrostatic attraction of electroactive transition metal complexes or organic dyes to oligonucleotide-DNA ds-complexes, e.g. $Co(bpy)_3^{3+}$, acridin or Hoechst 33258 were reported[6-10]. Microgravimetric quartz-crystal-microbalance, QCM[11] analyses were also applied to sense the formation of complementary oligonucleotide-DNA complexes.

Two major difficulties are still encountered in the development of DNA sensors and relate to the sensitivity and specificity of the resulting sensing systems.

GENERAL DESCRIPTION OF THE INVENTION

It is an object of the invention to provide a method and system for detecting target oligonucleotides in a sample.

The term *"detect"* or *"detection"* refers collectively to both a qualitative determination of the presence of the target oligonucleotide in the sample as well as at times for evaluation of the level of the target oligonucleotide in the sample.

In accordance with the first aspect of the invention there is provided a method for detecting a target oligonculeotide in a sample, comprising:

(a) providing a sensor device having a sensing interface carrying capturing oligonucleotides having each a nucleotide sequence complementary in at least a portion thereof to a first portion of the target oligonucleotides;

(b) providing verification oligonucleotides having each a nucleotide sequence complementary in at least a portion thereof to a second portion of the target oligonucleotide, other than said first portion;

(c) contacting the sample with the sensing interface under conditions such so as to allow the target oligonucleotides, if present in the sample, to hybridize to the capturing oligonucleotides;

(d) prior to (c) or thereafter, allowing the verification oligonucleotides to hybridize to the target oligonucleotides if present in the sample; and

(e) detecting the presence of said verification oligonucleotides on the sensing interface.

In accordance with another aspect, the present invention provides a system for detecting a target oligonucleotide in a sample, comprising:

(i) a sensor device having a sensing interface carrying capturing oligonucleotides having each a nucleotide sequence complementary in at least a portion thereof to a first portion of the target oligonucleotides;

(ii) verification oligonucleotides having each a nucleotide sequence complementary in at least a portion thereof to a second portion of the target oligonucleotide, other than said first portion; and

(iii) a combination comprising one or both of apparatus and reagents for detecting a verification oligonucleotide bound to the sensing interface.

The sample may be a biological specimen or a fractionation product thereof containing the oligonucleotides; a biological specimen treated to free and solubilized oligonucleotides; a specimen treated in a manner so as to digest nucleotide sequence into smaller oligonucleotides; a sample of oligonucleotides obtained by a PCR (Polymer Chain Reaction) process or any other oligonucleotide amplification process; etc.

In accordance with one embodiment, the present invention may be applied for a variety of genetic screening assays, such as, for example, screening intended to locate mutant genes.

In accordance with another embodiment, the invention may be applied for identifying pathogens in a sample.

There is a wide variety of assaying techniques available for detecting oligonucleotides which are based on hybridizing a probe oligonucleotide to the target oligonucleotide. Also known are assay techniques wherein a probe oligonucleotide is bound to a solid support which hybridize and *"fish out"* the target oligonucleotide from a tested sample. The invention is however unique in that it makes use of a verification oligonucleotide which increases both specificity and sensitivity of the assay.

In accordance with the invention, the verification oligonucleotide serves as an indicator for the presence of the target oligonucleotide in the

sample. In other words, detection of an immoblized verification oligonucleotide on the surface is an indication that the target oligonucleotide is bound to the sensing surface and hence that it existed in the sample. In accordance with the invention there are thus two discrimination means to ensure specificity and sensitivity:

1. Hybridization of the target oligonucleotide to the capturing oligonucleotide on the sensing surface. The complementary sequence of the capturing oligonucleotide will typically, but not exclusively, comprise of a number of oligonucleotides completing about one helix of the nucleotide strand, i.e. about twelve nucleotides. On the one hand, a complementary sequence of twelve oligonucleotides ensure on the one hand stable hybridization. On the other hand, a 12-mer oligonucleotide decreases the chance of binding to an incorrect oligonucleotide than a longer sequence. In the case the sample is a digested specimen of genomic DNA, or a fractionation product thereof comprising the oligonucleotides, there is some probability, which increases with the length of the capturing oligonucleotide, of binding to an incorrect oligonucleotide, namely an oligonucleotide other than the target oligonucleotide. This probability is lower, as aforesaid in the case of a shorter oligonucleotide. A sequence of about 12 nucleotides is preferred as it is optimal as far as ensuring binding stability, on the one hand, and reducing incorrect binding on the other hand. The invention is, however, not limited to such a capturing oligonucleotide.

2. Hybridization of the verification oligonucleotide to the target oligonucleotide.

These two independent binding events thus reduce the chance of false positive or false negative results.

The detection of the verification oligonucleotide on the sensing surface may be achieved by a number of means. In accordance with one embodiment of the invention, the sensor device comprises an electrochemical probe, e.g. for Faradaic impedance spectroscopy measurement or amperometric detection of the oligonucleotide. In addition, detection may also be carried out by a number of other electrochemical techniques known *per se* based on the control of interfacial electron transfer rates between the sensing interface and the surrounding medium. For this electrochemical embodiment of the invention, the sensing surface is formed on a conductive matrix on which the capturing oligonucleotides are bound. Such an electrically conducting matrix may for example be made or coated by a metal such as gold, platinum, silver or copper.

In accordance with another embodiment of the invention, the sensing device is a quartz crystal microbalance (QCM) probe in which case the presence of the verification oligonucleotide on the sensing surface is based on measurement of changes in resonance frequency of the probe. Microgravimetric QCM techniques are known *per se*, and are described, for example, in PCT Application WO 97/04314[(12)].

In accordance with one preferred embodiment the verification oligonucleotide are conjugated to a recognition agent which specifically binds to a signal-amplifying agent.

The recognition agent may for example be biotin, a ligand or an antigen, whereas the signal-amplifying agent may respectively comprise avidin or streptavidin, a soluble receptor or an antibody (the term *"antibody"* should be understood as referring to a polyclonal or monoclonal antibody, to a fraction of an antibody comprising the variable, antigen-binding portion, etc.).

At times, the signal-amplifying agent may be a complex which comprises a specific binding partner to the recognition agent which is bound or complexed to another agent. Such other agent may include, for example, colloid particles, super molecular structures, etc. In addition, the signal-amplifying agent may also be conjugated to or complexed with a label including, but not limited to an enzyme label. In case of an enzyme label, the enzyme is of a kind that can catalyze a reaction giving rise to an insoluble product. In accordance with this embodiment, the enzyme, after the signal-amplifying agent binds to the recognition agent, is allowed to catalyze the reaction which gives rise to the insoluble product, and said product then precipitates on to the sensing surface. This product may then be detected by a variety of electric/electronic or optical detection means. An assay carried out in accordance with the electrochemical embodiment, such a precipitate is preferably detected by the large change in electrode impedance resulting therefrom or a mass change on a piezaelectric crystal resulting in a frequency change of the crystal.

The invention will now be described with reference to a non-limiting specific embodiment. As will no doubt be appreciated, this description is a mere illustrative example of the wider scope of the invention as defined in the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates a bioelectronic system in accordance with an embodiment of the invention including assembly of the sensor and its use in detection of an target oligonucleotide in a sample.

Figs. 2A-2D illustrate in somewhat more details some of the components of the system of Fig. 1 as used in the exemplary experiments: Fig. 2A shows a DNA strand covalently bound to biotin; Fig. 2B illustrates the chemical reaction, catalyzed by horseradish peroxidase (HRP), in which

4-chloro- naphthol is reacted to form an insoluble product; Fig. 2C shows the structure of the thiophosphate thymine; and Fig. 2D shows the sequence of some oligonucleotides used in the exemplary experiments.

Figs. 3A and 3B show the impedance features, presented as Nyquist plots, of a bare electrode (curve a), after functionalization of the electrode with the capturing oligonucleotide (curve b), after binding the target DNA and the biotinylated oligonucleotide hybrid (curve c), after interaction with the avidin-HRP conjugate (curve d) and after some period of catalysis of the enzyme resulting in deposit of insoluble product on the sensing surface (curve e). It should be noted that Figs. 3A and 3B are of the same experiment but drawn to different scales.

DETAILED DESCRIPTION OF A PREFERRED EMBODIMENT

The manner of assembly of the DNA sensor in accordance with an embodiment of the invention and its use, are illustrated in Fig. 1. Oligonucleotide **100**, serving as the capturing oligonucleotide, includes a first portion **102**, typically a 12-based sequence, that is complementary to a first portion of the target oligonucleotide, and a second portion **106** for binding to the electrode, e.g. a gold (Au) electrode **108**. The binding portion **106** may, for example, be a several base (e.g 5) thiosphosphate thymidine (TS) sequence, illustrated in Fig. 2C. Occasionally the two portions **102, 106** may be separated by one or more separator base-nucleotides. The electrode **108** and oligonucleotide **100** are reacted such that portion **106** binds to the surface of the Au electrode. Thus, a functionalized electrode with a sensing surface **110** is thus formed.

A verification oligonucleotide **112** is contacted with a sample which contains the target oligonucleotide **104** whereby a partial double-stranded structure **114** is formed. This structure is then contacted with the sensing surface (step B) yielding a bifunctional double-stranded oligonucleotide

assembly 116. It should be noted that it is possible in accordance with another embodiment of the invention to first contact the sensing surface 110 with the sample and only then bring a reagent solution which comprises the verification oligonucleotide 112 into contact with the sending surface. This will first yield a binding of the target oligonucleotide 104 (if present in the sample) to the sensing surface and then binding of verification oligonucleotide 112 to yield assembly 116. In both cases. the presence of the verification oligonucleotide 116 on the sensing surface serves as an indication of the presence of the target oligonucleotide 104 in the sample.

The detection of the verification oligonucleotide on the sensing surface may be achieved by a number of means some of which were explained above. For example, the verification oligonucleotide may carry a label which may be detected electrically, e.g. by determining change in impedance, or electron transport between the electrode 108 and the surrounding medium. The label, by one embodiment, is an enzyme which can catalyze a reaction yielding an insoluble reaction product which precipitates on the surface's electrode thus increasing impedance. This is illustrated in step C of Fig. 1.

In the specific illustrated embodiment, verification oligonucleotide 112 is bound to a biotin moiety 117. A label complex 118 which comprises an avidin 119 bound to an enzyme 120 is contacted with the sensing surface (step C) resulting in binding of complex 118 to the sensing surface. Enzyme 120 can catalyze a reaction converting a substrate (S) into an insoluble product (P) which is thus deposited on the sensing surface. Both the binding of the labeling complex 118 to the sensing surface as well as the precipitation of product (P) onto the sensing surface can be monitored similarly as above (i.e. change of impedance or a change of mass in the case of QCM-type measurement).

The invention will now be further illustrated by the following example:

Example

For clarity, in the description below the same reference numeral to those used above will be used.

It should be noted that the scheme shown in Fig. 1 can be employed for various different assays than that specifically exemplified herein. Furthermore, a similar scheme, *mutatis mutandis*, may also be used for assaying a target oligonucleotide in other assay techniques, e.g. microgravimetric QCM. In this latter case rather than electric/electronic measurements, the measurement is of change in resonance frequency of the piezoelectric crystal as a result of mass change.

An 18-mer oligonucleotide **100** which included a 12-base sequence **102** that is complementary to a part of the analyte, the Tay-Sachs (TS) mutant **104** was used. In addition, oligonucleotide **100** included a 5-base thiophosphate thymine-TS tag **106** for its assembly on the gold (Au) electrode **108**, and a single T-base separating the tag from the sensing oligonucleotide sequence. A disc Au-electrode **108**, 0.05 cm^2, was interacted with oligonucleotide **100** (20 μM, 10 hours) resulting in the assembly of the tensing interface on the gold support (step A in Fig. 1). The resutling functionalized electrode **110** was interacted with a solution that included the target analyte, the TS-mutant sequence **104** (5.8×10^{-7} g/mL^{-1}, 4 hours), and a biotinylated verification oligonucleotide **112**, 2×10^{-5} g/mL^{-1} (step B in Fig. 1).

Verification oligonucleotide **112** is complementary to one portion of an oligonucleotide **104** and consequently these two oligonucleotides hybridize to form a partial double-stranded structure **114**. Target oligonucleotide **104** has another sequence complementary to portion **102** of capturing oligonucleotide **100** and thus step B results in the formation of a bifunctional double-stranded DNA-oligonucleotide assembly **116**.

Sensing surface with bifunctional double-stranded DNA-oligonucleotide assembly **116** is then treated with an avidin labeled with horseradish peroxidase (HRP) (1×10^{-8} g/mL^{-1}, 3 hours) (step C in Fig. 1). HRP can catalyze the oxidation of 4-chloro-1-naphthol (S) by hydrogen peroxide (H_2O_2) giving rise to the formation of an insoluble product (P) which precipitates on the electrode. Other enzyme-substrate couples yielding an insoluble product which may be used include: alkaline phosphatase and indoyl phosphate derivatives as substrates; glucoze ozidase and tetrazolium salts as substrates; etc.

As the oligonucleotide and oligonucleotide-DNA layered assemblies are negatively charged, the electrostatic repulsion of a negatively-charged redox-probe, e.g. $Fe(CN)_6^{3-/4-}$, from the electrode support is anticipated to perturb the interfacial electron transfer. This is expected to introduce an electron transfer resistance that can be detected by Faradaic impedance spectroscopy or other electrochemical means such as reduction of the amperometric response of the electrode. The biocatalytic precipitation of the product (P) on the electrode is expected to further insulate the conductive support and to lead to a high interfacial electron transfer resistance or a reduction of the amperometric response of an electroactive species solubilized in the medium surrounding the electrode.

Fig. 3A shows the impedance features, using $Fe(CN)_6^{3-/4-}$ as redox-probe, presented as Nyquist plots (Z_{im} vs. Z_{re}), of the bare electrode **108** (curve a), of the functionalized electrode with the sensing surface **110** (curve b) and of the layered bifunctional double-stranded oligonucleotide-target DNA and biotinylated oligonucleotide assembly (curve c). The respective semicircle diameters correspond to the interfacial electron transfer resistnaces, R_{et}. It can be seen that the electron transfer resistance increases upon the build-up of the biotinylated oligonucleotide-DNA assembly. For example, for the functionalized electrode R_{et} = 1.1 kΩ whereas R_{et} is increased to about

2.2 kΩ upon the association of the complex **114**. These results are consistent with the fact that the negative charge increases upon the two-step organization of the assembly. This results in the enhanced electrostatic repulsion of the redox-probe, and introduces higher interfacial electron transfer resistances.

Fig. 3B shows the impedance spectra of the bifunctional double-stranded assembly consisting of the target DNA linked to the sensing interface and the biotinylated oligonucleotide, before (curve c) and after (curve d) interaction with the avidin-HRP conjugate. Upon the association of the avidin-HRP biocatalytic conjugate to the layer, a considerable increase in the electron transfer resistance is observed due to the partial insulation of the electrode by the proteins. In the presence of H_2O_2 and the substrate (S), the biocatalytic precipitation of the product onto the electrode occurs. This insulates the conductive support, resulting in a very high increase in the electron transfer resistance, curve (e), R_{et} = 17 kΩ. It should be noted that the two parameters controlling the sensitivity of the DNA-sensing devices are the time of incubatin of the functionalized-monolayer-electrode **110** with the complex **114** and more important, the time-interval used to precipitate the product by the avidin-HRP biocatalyic conjugate. Using this configuration, and upon precipitation of P for 40 mins. it was possible to sense the target DNA **104** at a concentration of 20×10^{-9} g/mL^{-1}, R_{et} = 7.9 kΩ.

Control experiments show that the oligonucleotide sensing assembly has a high specificity and selectivity. Treatment of the functinalized electrode **110** with the biotinylated oligonucleotide **112** and then with the avidin-HRP conjugate **118**, but without the interaction with the target DNA **104**, yielded only a minute change in the electron transfer resistance.

In order to test the specificity of the system, the same assay was performed with a DNA fragment **104'** that corresponds to the normal gene sequence in which the 7-based mutation leads to the TS-genetic disorder. After contact of the sensing interface with a complex between fragement **104'**

and the verification oligonucleotide **112**, the system was subjected to the biocatalytic precipitation process using the avidin-HRP conjugate, using the same protocol as illustrated in Fig. 1. However, no noticeable changes in the electron's transfer resistance at the electrodes were observed, implying that the lack of formation of a complex between the capturing oligonucleotide on the sensing surface and the complex formed between the target oligonucleotide **104** and the verification oligonucleotide 112 which prevented the subsequent formation of the precipitant layer on the electrode.

Cyclic voltammetry experiments (see insert Fig. 3B) further confirm the stepwise organization of the bifunctional double-stranded complex **116**, and that the precipitation of the insulating layer formed by product P on the electrode, gradually perturb the electron-transfer kinetics of $Fe(CN)_6^{3-}$. Fig. 3B inset, shows the cyclic voltammograms of $Fe(CN)_6^{3-}$ at a bare Au-electrode (curve a), upon formation of the sensing assembly **110** (curve b), and upon the formation of the double-stranded assembly **110** (curve c). The stepwise assembly of the layers is accompanied by a decrease in the amperometric response of the electrode and an increase in the peak-to-peak separation between the cathodic and anodic waves of the redox-probe. This is consistent with the enhanced electron transfer barriers introduced upon the assembly of the negatively-charged oligonucleotide assembly. Association of the avidin-HRP conjugate onto the layer (curve d), further separates the redox waves of $Fe(CN)_6^{3-}$ implying that binding of the protein insulates the electrode and perturbs the interfacial electron transfer. Biocatalytic precipitation of **P** onto the electrode insulates the conductive support, and the electrical response of the redox-probe is almost entirely blocked, (curve e). The result shown in the inset of Fig. 3B demonstrates that amperometric transduction of the formation of the complex **116**, binding of avidin-linked HRP **118**, and further precipitation of the product **P** is possible.

By some modification of the assayed scheme described above, rather than determining the formation of the insoluble product precipitates on the electrode by means of a Faradaic impedance spectroscopy, it may also be determined by means of amperometric detection, by microgravimetric QCM detection, by optical means and others.

CLAIMS:

1. A method for detecting a target oligonucleotide in a sample, comprising:

(a) providing a sensor device having a sensing interface carrying capturing oligonucleotides having each a nucleotide sequence complementary in at least a portion thereof to a first portion of the target oligonucleotides;

(b) providing verification oligonucleotides having each a nucleotide sequence complementary in at least a portion thereof to a second portion of the target oligonucleotide, other than said first portion;

(c) contacting the sample with the sensing interface under conditions such so as to allow the target oligonucleotides, if present in the sample, to hybridize to the capturing oligonucleotides;

(d) prior to (c) or thereafter, allowing the verification oligonucleotides to hybridize to the target oligonucleotides if present in the sample; and

(e) detecting the presence of said verification oligonucleotides on the sensing interface.

2. A method according to Claim 1, wherein the sensor device comprises an electrochemical probe carrying the sensing interface.

3. A method according to Claim 2, wherein said detection is based on Faradaic impedance spectroscopy or amperometric measurements.

4. A method according to any one of Claims 1-3, wherein the sequence complementary to at least a portion of the target oligonucleotide of about 12 nucleotides.

5. A method according to any one of Claims 1-4, wherein the verification oligonucleotide is conjugated to a recognition agent which specifically binds to a signal-amplifying agent.

6. A method according to Claim 5, wherein said recognition agent is biotin and said signal amplifying agent comprises avidin.

7. A method according to Claim 5, wherein said signal-amplifying agent comprises an enzyme which can catalyze a reaction yielding an insoluble reaction product.

8. A system for detecting a target oligonucleotide in a sample, comprising:

(i) a sensor device having a sensing interface carrying capturing oligonucleotides having each a nucleotide sequence complementary in at least a portion thereof to a first portion of the target oligonucleotides;

(ii) verification oligonucleotides having each a nucleotide sequence complementary in at least a portion thereof to a second portion of the target oligonucleotide, other than said first portion; and

(iii) a combination comprising one or both of apparatus and reagents for detecting a verification oligonucleotide bound to the sensing interface.

9. A system according to Claim 8, wherein said sensor device is an electrochemical electrode carrying said sensing surface.

10. A system according to Claim 8 or 9, wherein said apparatus is adapted for the performance of an electrochemical measurement.

11. A system according to any one of Claims 8-10, wherein said capturing oligonucleotide has a nucleotide sequence complementary to said first portion which has a length of about 12 nucleotides.

12. A system according to any one of Claims 8-11, wherein the verification oligonucleotide is conjugated to a recognition agent which specifically binds to a signal-amplifying agent.

13. A system according to Claim 12, wherein said recognition agent is biotin and said signal-amplifying agent comprises avidin.

14. A system according to Claim 14, wherein the avidin is bound to an enzyme which can catalyze reaction yielding an insoluble reaction product.

For the Applicants,
REINHOLD COHN AND PARTNERS
By:

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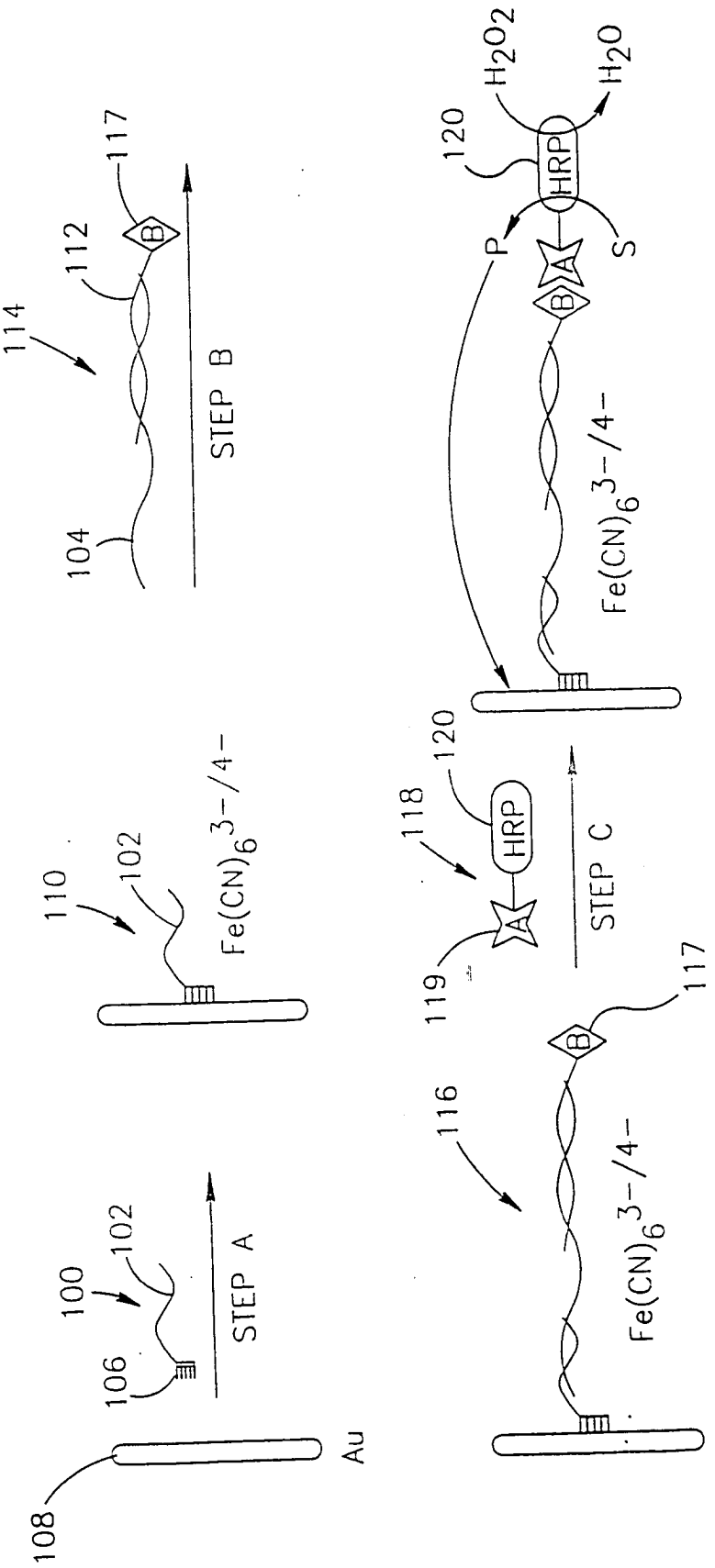

108
Au
100
102
106
STEP A
110
102
$Fe(CN)_6^{3-/4-}$
114
112
117
B
104
STEP B
118
120
HRP
119
A
STEP C
116
B
117
$Fe(CN)_6^{3-/4-}$
120
H_2O_2
H_2O
HRP
P
S
B
A
$Fe(CN)_6^{3-/4-}$

FIG. 1

B=BIOTIN

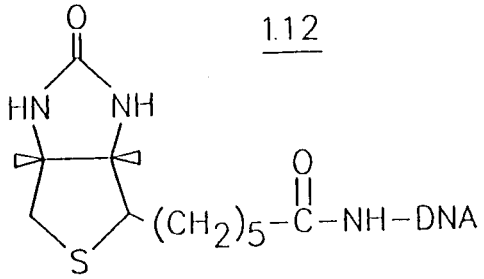


FIG.2A

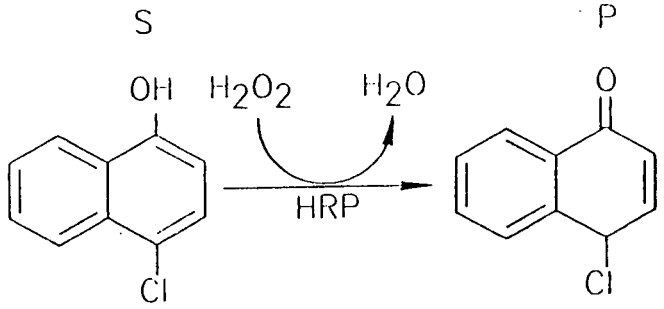


FIG.2B

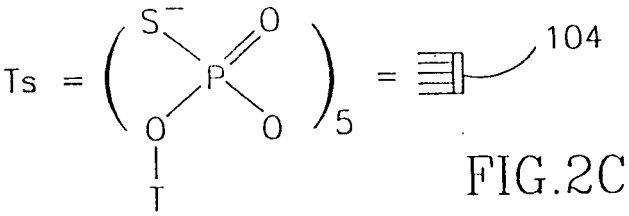


FIG.2C

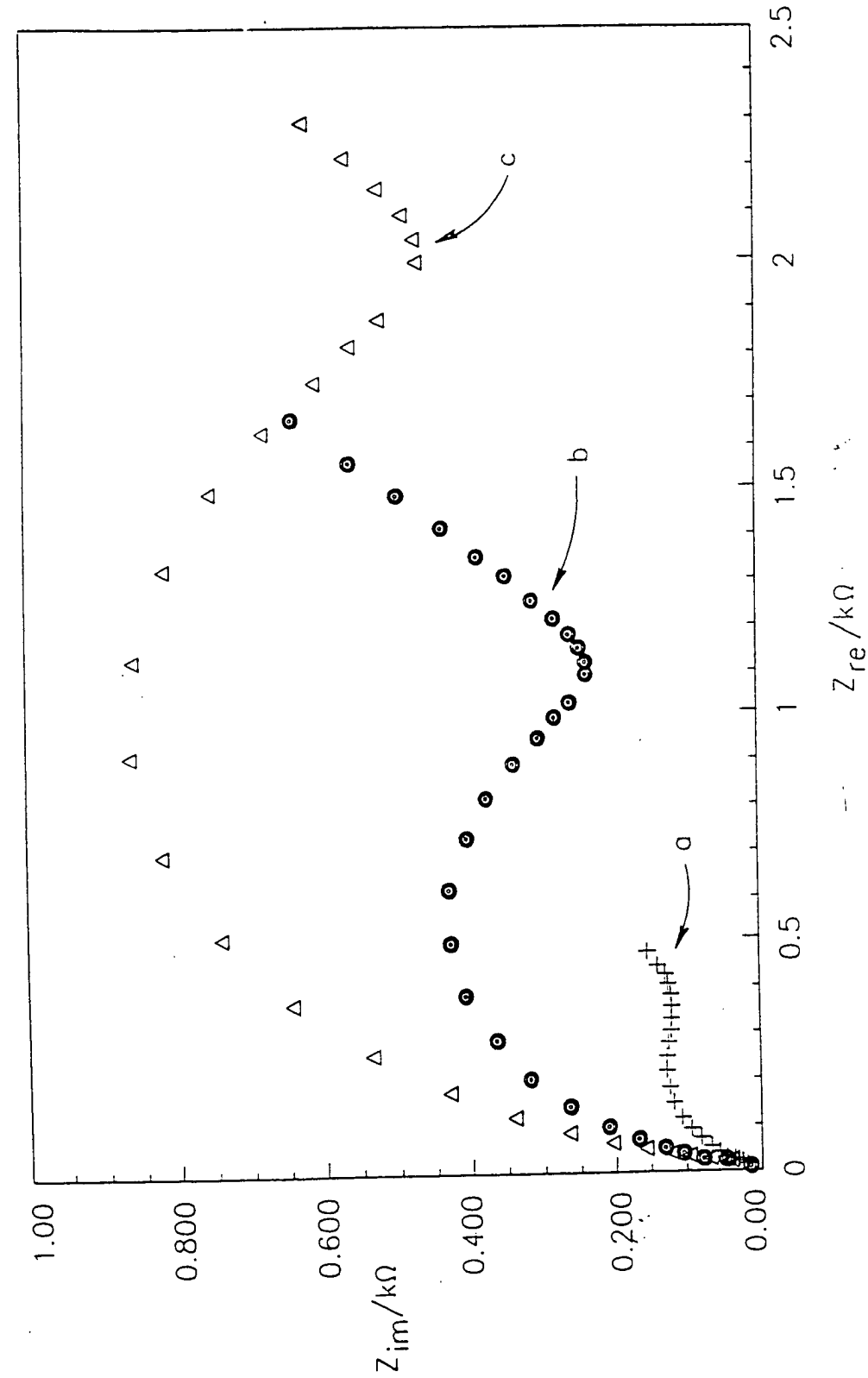
100
$3'_{TsTsTsTsTsTCGCATCCTATCT}5'$

112
$3'_{ATATGCCAAGCGCG}5'$ –BIOTIN

104
$5'_{GCGTAGGATAGATATACGGTTCGCGC}3'$

104'
$5'_{GCGGCCATAGGATATACGGTTCGCGC}3'$

FIG.2D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0.800
0.600
0.400
0.200
0.00
$Z_{im}/k\Omega$
0
0.5
1
1.5
2
2.5
$Z_{re}/k\Omega$
a
b
c

FIG.3A

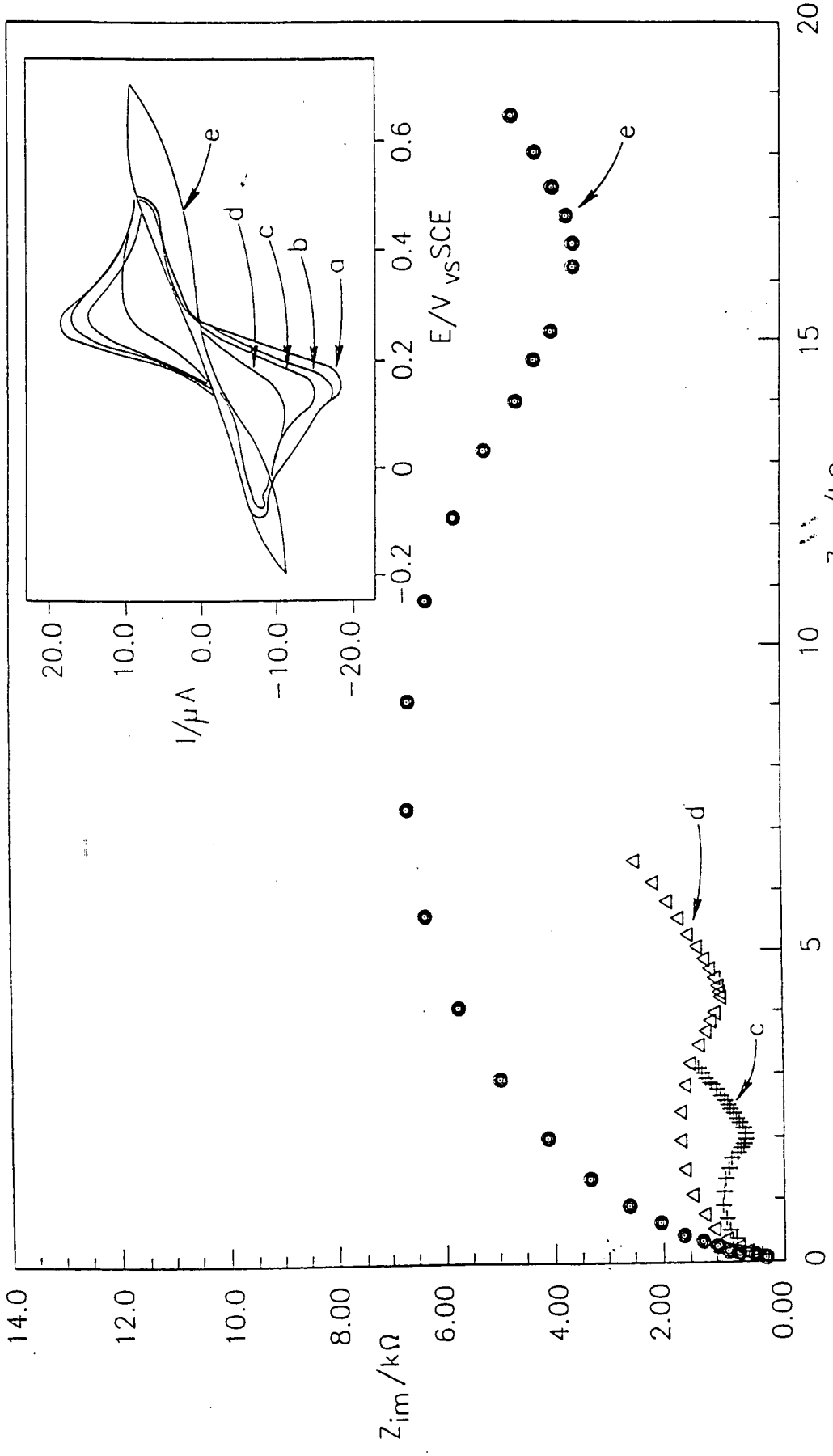

FIG.3B